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Use of Thai local vegetable extracts as natural preservatives in dried sausage system

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ABSTRACT: Antioxidant and antimicrobial activities of Thai local vegetable extracts (three formulations) at the concentration of 0.02%, 0.1% and 0.2% in a Chinese-style sausage during storage at 4°C and 85% relative humidity (RH) for 20 days were evaluated. One of the three formulations of the extracts were *Polygonum odoratum* extract (PE) while the other two were mixed vegetable extracts of formulation 1 (MVE_1) and formulation 2 (MVE_2) which were the mixture of *P. odoratum, Cassia siamea, Garcinia cowa* and *Limnophila aromatic* extracts. All formulations of these vegetable extracts were able to delay lipid oxidation in the sausage. Addition of 0.2% vegetable extracts resulted in a greater decrease of TBARS value as compared to the other concentrations, but caused unacceptable color of the sausage. The vegetable extracts at 0.1% were selected for use in combination with 2.5% sodium lactate (SL) as preservatives in this sausage. Their effects on oxidative, microbial and sensory stability of sausage samples during storage at 4°C and 85% RH for 21 days were evaluated. These extracts were able to retard lipid oxidation by lowering TBARS value throughout the 21-day storage. Addition of 2.5% SL, either alone or in combination with 0.1% of these natural extracts resulted in decreasing number of total microbial counts in the samples. The total microbial counts in the samples added with SL alone, PE with SL, and MVE_2 with SL decreased by 1.36, 1.35, and 2.42 log units after 21 days of storage. However, addition of each vegetable extract alone did not result in reduction of total microbial counts in the sausages during storage. The sausage samples added with PE in combination with SL had less rancid odor compared to the samples of other treatments. Therefore, the vegetable extract, PE in combination with SL could be used to extend shelf-life of this sausage.

KEYWORDS: antioxidant, antimicrobial, *Polygonum odoratum*, sodium lactate

INTRODUCTION

Oxidative rancidity and microbial spoilage result in an increase of off-flavors and a decrease of nutritional quality and safety of a high-fat intermediate moisture or dried meat products. These products made with a normal recipe usually contain a high amount of fat. Normally, pork back fat used in these sausages has more than 60% unsaturated fatty acid content. Most of the products are stored in air-permeable packages.[1] During storage, the relatively high proportion of unsaturated fatty acids has potential for decomposing by oxidation processes. One of the main reactions of lipid oxidation is autooxidation. The autooxidation process proceeds in three steps: initiation, propagation and termination. This reaction is implicated in the development of off-flavors, loss of meat color and the formation of harmful lipid oxidation products. Hydroperoxides formed in the initial stage of oxidation are relatively unstable. They subsequently decompose to form volatile aroma compounds, perceived as off-flavors. Oxidation of the muscle pigment oxymyoglobin to metmyoglobin also leads to the discoloration of red meats.[2–3] Moreover, microbial deterioration of these meat products also affects their quality and safety.

Use of antioxidant and antimicrobial agents is an effective way to minimize or retard the lipid oxidation and microbial spoilage in the high-fat meat products. In recent years, natural preservatives from plants have received much

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interest to apply in meat products due to an increase in consumer awareness of the synthetic preservative side effects.[4] Some researchers have reported the effectiveness of some plant extracts as natural antioxidants and antimicrobials in meat products such as the extracts of chamnamul (*Pimpinella brachycarpa*) and fatsia (*Aralia elata*) in ground beef patties[5] and red grape pomace extracts in pork burgers.[4] These effects may result from the action of active compounds in these plant extracts. Some active compounds in plants have been identified such as sulfated, methylated and glycosidal flavonoids from *Polygonum hydropiper*, a medicinal herb used in Japanese cuisine[6] and flavonoids (rutin, catechin, quercetin, kaempferol and isorhamnetin) in *Polygonum odoratum* leaves.[7]

Some species of green vegetable extracts have been reported to possess both antioxidant and antimicrobial activities.[5, 7] Four Thai local vegetable extracts including the extracts of Thai copper pod (*Cassia siamea*), chamuang (*Garcinia cowa*), finger grass (*Limnophila aromatica*) and Vietnamese coriander (*P. odoratum*) were reported to possess antimicrobial and antioxidant properties.[7] Thus, it may be possible to use these vegetable extracts as natural preservatives in high-fat meat products. However, the use of these natural preservatives in combination with sodium lactate, a weak organic acid salt can represent an ideal application of the 'multiple hurdle' concept in foods. Several combined hurdles have the potential to increase the microbiological safety and shelf-life of food products.[8] Sodium lactate exerts antimicrobial activity and has been traditionally used as a preservative in several types of meat products. This weak organic acid salt is a generally recognized as safe (GRAS) substance and has been approved as food additives by E.C., FAO/WHO and FDA.[9] Therefore, the objectives of this study were to investigate the effectiveness of Thai local vegetable extracts either alone or in combination with sodium lactate on delaying lipid oxidation, reducing microbial growth, color change and rancid odor in a Chinese-style sausage.

MATERIALS AND METHODS

Production of natural additives

Plant materials including fresh leaves of four Thai local vegetables, *Cassia siamea* Britt. (Thai copper pod, a common name), *Garcinia cowa* Roxb. (chamuang), *Limnophila aromatica* Merr. (finger grass) and *Polygonum odoratum* Lour. (Vietnamese coriander) as well as dried rosemary leaves (*Rosmarinus officinalis'* L), a positive control plant, were purchased at retail outlets in Bangkok, Thailand. They were cut into small pieces, freeze-dried and powdered. Then, 10 g of each were soaked in 150 ml methanol, and was shaken at 200 rpm for 24 hrs at ambient temperature. The mixtures were filtered. The filtrates were evaporated using vacuum rotary evaporator and air dried at 40°C. Dried extracts were mixed with maltodextrin (DE value = 17–19%) as a carrier in the ratio of 40:60 (plant extract: maltodextrin, % w/w) to achieve different formulations of dried plant extracts including: PE (*P. odoratum* extract only), MVE_1 (mixed vegetable extracts 1), MVE_2 (mixed vegetable extracts 2) as well as RM (Rosemary extract only). MVE_1 and MVE_2 contained mixture of *C. siamea, G. cowa, L. aromatica* and *P. odoratum* extracts in the ratio of 10:10 5:15 and 10:5 10:15 (a total of 40%w/w), respectively. All ingredients were mixed and dried in a freeze dryer (Labconco, Bio. 31/02, Kansas city, USA).

Application of vegetable extracts as natural preservatives in the sausage

Effect of plant extracts at different concentrations on oxidative and microbial stability of the sausage

First of all, the effect of 0.02%, 0.1% and 0.2% plant extracts (PE, MVE_1, MVE_2 and RM) on lipid oxidation and microbial growth in the Chinese-style sausage was studied. The sausage was manufactured according to a conventional formula: 62.3% ground pork, 23.36% pork backfat, 9.97% sugar, 0.62% salt and 3.74% water. All ingredients were mixed together using a bowl mixer (Kitchen aid, Large Appliances; Benton Harbor, MI, USA) and evenly divided into 14 parts (400 g each) for addition of different additives at each concentration. Each portion of sausage mixture was added with either one of 0.02%, 0.1% and 0.2% PE, MVE_1, MVE_2, RM and 0.02% butylated hydroxytoluene (BHT) (positive controls). The sausage mixture was filled into natural pork casing. The sausages were dried in a hot-air oven at 43°C for 48 h before storing at 85% relative humidity, 4°C for 20 days. The samples were analyzed for the thiobarbituric acid reactive substance (TBARS) values at day 0, 5, 12, 16 and 20 of storage. The total microbial counts, pH, a_w at 30°C were determined at day 0 and day 20 of storage. The appropriate concentration of these natural additives was selected for use in the next step of the experiment.

Combined effect of plant extracts and sodium lactate on retardation of lipid oxidation and microbial growth in the sausage

In this experiment, natural additives (PE, MVE_1, MVE_2 and RM) at the selected concentration were used in combination with sodium lactate (SL) as preservatives in the same sausage. To prepare this sausage, all ingredients (a total weight of 6,050 g) were mixed and evenly divided

into 11 parts (550 g each). Before stuffing into the natural pork casing, the additives (natural or synthetic) were added and thoroughly mixed: treatment 1, control (without additive); treatment 2, 0.02% BHT; treatment 3, 2.5% SL; treatment 4, 0.1% PE; treatment 5, 0.1% PE and 2.5% SL; treatment 6, 0.1% MVE_1; treatment 7, 0.1% MVE_1 and 2.5% SL; treatment 8, 0.1% MVE_2; treatment 9, 0.1% MVE_2 and 2.5% SL; treatment 10, 0.1% RM and treatment 11, 0.1% RM and and 2.5% SL. The sausages were dried in the hot-air oven at 43°C for 48 h before storing at 85% relative humidity, 4°C for 21 days. Total microbial counts, TBARS, pH and color values of the samples were analyzed at day 0, 3, 7, 14 and 21 of storage. Sensory evaluation was performed at the end of the storage. Some analysis methods are described in more details as follows.

Analysis of the samples

Microbiological analysis

The total microbial counts in the sausage samples were determined according to the method described by Maturin and Peeler.[10] To determine the total microbial counts, 25 g of the sample were homogenized with 225 ml of 0.1% sterile peptone water by using a stomacher, and subsequently serially diluted in 0.1% sterile peptone water. Then, they were plated out onto plate count agar by spiral plating technique using Automated Spiral Plater (Autoplate® 4000, Spiral Biotech, Norwood, MA, USA). All plates were incubated at 37°C for 24 hrs.

Analysis of Thiobarbituric acid reactive substances

To determine the extent of lipid oxidation, TBARS values of the sausage samples were analyzed using the method described by Tarladgis et al.[11] The sample (10 g) was macerated with 50 ml distilled water. The mixture was transferred with an additional 47.5 ml distilled water rinsed into a distillation flask. Then, 2.5 ml of 4 M HCl and a few glass beads were added into the same distillation flask. The mixture was distilled to collect 50 ml distillate. Five mililiters of the distillate were transferred into a glass-stoppered tube and subsequently added with 5 ml thiobarbituric acid (TBA) reagent (0.2883 g/100 ml 90% glacial acetic acid). The tube was shaken and heated in boiling water bath for 35 min to develop the chromogen. A blank was similarly prepared by using 5 ml distilled water with 5 ml TBA reagent and followed the same procedure. Then, tubes were cooled in cold water for 10 min. The absorbance was measured against blank at 538 nm using spectrophotometer (SHIMADZU, UV-1601, Japan). The TBARS values (mg malonaldehyde (MDA)/kg sample) were calculated by multiplying the absorbance values by K value. The K value was calculated using malonaldehyde standard curve.[11–12] 1,1,3,3-tetraethoxypropane (Sigma Aldrich Inc., Germany) was used to prepared a standard solution.

Color measurements

Color of the sausage samples was measured using a Konica Minolta CR-300 Chroma Meter. Color values were described as L*, brightness; a*, redness (+)/greenness (−); b*, yellowness (+)/blueness (−).

Sensory evaluation

The samples of all treatments were evaluated by seven trained taste panels using descriptive analysis (scoring). The same person evaluated the samples at the beginning and the end of the storage period. The questionnaires measured intensity on a 5-point scoring (weak to strong) for these following attributes: color, texture, rancid flavour, sweetness and overall acceptability.

Statistical analysis

Data of three replications were analyzed by using analysis of variance to determine if significant differences ($P \leq 0.05$) existed between mean values and using Duncan multiple range test to compare between treatment means.

RESULTS AND DISCUSSION

Antioxidant and antimicrobial effect of natural preservatives at different concentrations

The TBARS values of all sausage samples increased as the storage time increased (Figure 1). The samples added with natural preservatives of Thai local vegetable extracts (PE, MVE_1 and MVE_2) at all concentrations tested had significantly lower TBARS values ($P<0.05$) than those of the control samples (no additive added) at each storage time (Figure 1a). At 20-day storage, the samples added with 0.02% BHT had the lowest TBARS values (0.61 mg malonaldehyde (MDA)/kg sample). Natural preservatives (PE, MVE_1, MVE_2 and RM) at 0.2% in the samples caused more slowly increasing of the TBARS values (Figure 1c), compared to the lower concentration (0.1%, Figure 1b). However, 0.2% of each natural additive resulted in unacceptable color of the sausage. Generally, the pH and a_w of the samples slightly increased as storage time increased (Table 1). The microbial counts remained in high number (10^7–10^9 CFU/g) at the end of storage (20 days). Therefore, 0.1% of each natural preservative was selected for use in combination with sodium lactate in this sausage in the next experiment.

Antioxidative effect of natural preservatives in combination with sodium lactate

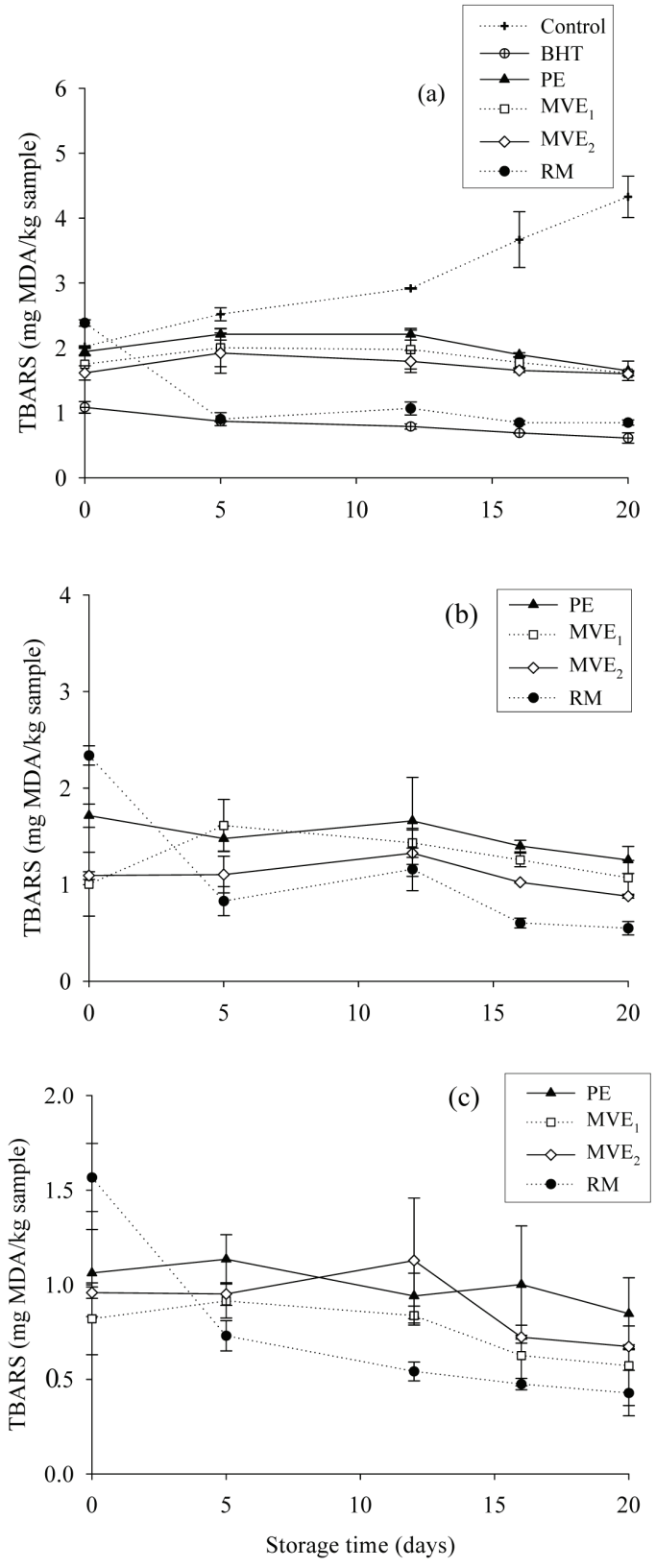

Figure 1: Change of TBARS values in the sausages added with some preservatives at the concentrations of 0.02% (a) 0.1% (b) and 0.2% (c) during storage at 4°C.

Table 1: Change of pH, water activity and total microbial counts in the sausages added with some preservatives at different concentrations during storage at 4°C

Treatments	a_w (30°C)[a]	pH values[a]	Total microbial counts (CFU/g)[a]
Control	0.81–0.88	5.16–5.57	2.1×10^8–1.9×10^9
BHT (0.02%)	0.78–0.83	5.23–5.65	9.9×10^5–6.1×10^7
PE (0.02%)	0.82–0.85	5.28–5.53	1.2×10^7–1.0×10^9
MVE_1 (0.02%)	0.82–0.86	5.31–5.53	1.2×10^7–1.1×10^9
MVE_2 (0.02%)	0.83–0.89	5.29–5.54	1.4×10^7–7.0×10^8
RM (0.02%)	0.83–0.88	5.17–5.47	2.0×10^7–1.9×10^9
PE (0.1%)	0.83–0.85	5.12–5.36	6.8×10^6–6.4×10^8
MVE_1 (0.1%)	0.81–0.85	5.23–5.37	8.5×10^5–8.1×10^8
MVE_2 (0.1%)	0.74–0.87	5.19–5.32	2.2×10^6–9.4×10^7
RM (0.1%)	0.74–0.86	5.15–5.38	1.7×10^7–8.4×10^8
PE (0.2%)	0.73–0.85	4.99–5.38	2.0×10^6–3.8×10^8
MVE_1 (0.2%)	0.83–0.85	5.12–5.31	1.5×10^6–3.9×10^7
MVE_2 (0.2%)	0.84–0.86	4.98–5.28	1.7×10^6–7.8×10^7
RM (0.2%)	0.83–0.88	4.99–5.30	1.2×10^7–2.7×10^8

[a]Data (at day 0-day 20) are mean of two replications.

The TBARS values in the samples of all treatments increased as storage time increased (Table 2). All sausage samples added with natural or synthetic preservatives had significantly lower TBARS values, compared to those of the control samples ($P<0.05$). Addition of *P. odoratum* extract (PE) and two mixed vegetable extracts (MVE_1 and MVE_2) in the sausage samples either alone or in combination with sodium lactate (SL) caused a slower increase of TBARS values, compared to the control samples and those added with SL alone. This indicated that addition of these natural additives could delay lipid oxidation in the samples. However, significant differences were not found between the TBARS values of the samples added with SL in combination with any of the natural extracts and those of the samples added with the natural extract alone ($P>0.05$). Addition of SL alone caused a slower increase of TBARS values as compared to these values in the control samples. Sallam[12] has previously reported that sodium lactate had some antioxidant activity.

The slow increase of TBARS values in the samples added with natural preservatives may be due to their antioxidant activity. Nanasombat and Teckchuen[7] reported that extracts of *P. odoratum, C. siamea, L. aromatica* and *G. cowa* had strong antioxidant activity. Among 20 species of Thai local vegetable extracts tested, *P. odoratum* extract had the strongest DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, with EC_{50} value of 315.4 µg extract/mg DPPH. Some organic compounds including ((*Z*)-3-hexenal, (*Z*)-3-hexenol, decanal, undecanal, and dodecanal) were found in *P. odoratum*.[13] Leaves and flowers of *C. siamea* contained an active compound, barakol.[14] Flavonoids, nevadensin 7-*O*-glycoside and 8-hydroxylated flavones were detected in *L. aromatica*[15] whereas five xanthones named cowagarcinone A-E were

Table 2: Change of TBARS values in the sausages added with some preservatives alone or in combination with sodium lactate during storage at 4°C

Treatments	TBARS[a] (mg MDA/kg) ± SD				
	0 day	3 days	7 days	14 days	21 days
Control	1.59±0.17A[b]	2.22±0.08A	2.67±0.32A	3.78±0.19A	3.97±0.09A
BHT (0.02%)	1.04±0.33B	0.98±0.25B	0.66±0.22C	0.45±0.01DE	0.34±0.03B
SL (2.5%)	0.96±0.03BC	1.85±0.12A	1.45±0.03B	1.43±0.16B	1.03±0.03B
PE (0.1%)	0.65±0.04BCD	1.01±0.13B	0.84±0.05C	0.66±0.03DC	0.57±0.04C
PE (0.1%) + SL (2.5%)	0.67±0.02BCD	1.08±0.19B	0.87±0.01C	0.74±0.01C	0.59±0.08C
MVE_1 (0.1%)	0.56±0.03CD	0.77±0.09B	0.67±0.02C	0.57±0.05DC	0.49±0.02DC
MVE_1 (0.1%)+ SL (2.5%)	0.58±0.17CD	0.98±0.03B	0.71±0.01C	0.61±0.03CDE	0.61±0.08C
MVE_2 (0.1%)	0.67±0.02BCD	0.83±0.06B	0.71±0.04C	0.63±0.06CDE	0.54±0.01C
MVE_2 (0.1%)+SL (2.5%)	0.63±0.18BCD	1.03±0.32B	0.86±0.15C	0.69±0.06C	0.62±0.05DE
RM (0.1%)	0.73±0.27BCD	0.87±0.18B	0.62±0.01C	0.43±0.09E	0.37±0.05DE
RM (0.1%)+ SL (2.5%)	0.42±0.17D	0.75±0.08B	0.64±0.04C	0.45±0.06DE	0.36±0.06DC

[a]Data are mean of two replications.

[b]Means within a column with different letters are significantly different ($P< 0.05$).

isolated from latex of *G. cowa*.[16] In this study, rosemary extract as well as BHT had strong antioxidative effect against lipid oxidation in the sausages. Risnar et al.[17] determined antioxidant and antimicrobial activities of rosemary extracts (Vivox 20 and Vivox 4 containing 20% and 4% carnosic acid as the main components, respectively) in chicken frankfurters and found that these rosemary extracts were able to delay lipid oxidation and inhibit microbial growth in the sausages. Bauman et al.[18] reported that rosemary contained monoterpenes (eteric oils), diterpene phenols (carnosic acid, carnosol, rosmanol, epirosmanol isorosmanol, methyl carnosate) phenolic acid (rosmarinic acid), flavonols and triterpene acids (ursolic acid, olemolic acid and butilinic acid).

Antimicrobial effect of natural additives in combination with sodium lactate

Total microbial counts in the sausages added with BHT, PE, MVE_1, MVE_2 and RM as well as the control treatment were in high number (7.24–8.35 log CFU/g) after 21 days of storage (Table 3). However, the addition of SL either alone or in combination with natural extracts (PE, MVE_1, MVE_2 and RM) in the sausages caused significantly lower microbial counts as compared to the samples without SL at each storage period ($P<0.05$).

Although the antimicrobial activity of these plant extracts has been reported,[7] the 0.1% concentration of each natural extract (PE, MVE_1, MVE_2 and RM) did not inhibit microbial growth in the sausage. High concentrations of these extracts may inhibit the growth of microorganisms in the sausage samples, but the addition of these extracts (more than 0.1%) may cause the unacceptable color and odor (red with green color and pungent odor) to occur. In the current study, the samples added with sodium lactate had lower microbial counts. These results were in agreement with those reported by Mbandi and Shelef.[19] They found that growth rate of *Listeria monocytogenes* and *Salmonella enteritidis* decreased in ground beef added with 1.8% sodium lactate and stored at 5°C for 20 days. In addition, Houtsma et al.[20] determined the minimum inhibitory concentration (MIC) of sodium lactate and found that addition of sodium lactate (no less than 268 mM) resulted in inhibition of bacterial growth at pH 5.7. Inhibitory effects of sodium lactate, a weak organic acid salt may be due to their undissociated form. The undissociated acid molecules could penetrate rapidly through cytoplasmic membrane, dissociate and acidify the cell interior. When the internal pH of cells decreases below a certain threshold value, cellular functions are inhibited.[21] Moreover, its chelating properties and ability to reduce the a_w may cause inhibition of microbial growth. Lactate is able to chelate a large portion of metallic nutrient ions, depleting the cell of its essential nutrients. In addition, sodium lactate dissociates into uncharged acid molecules, anions and cations. Accumulation of anion is responsible for the toxic effect of acids at low pH.[22]

Water activity, pH and color changes

The initial a_w of all treatment sausages ranged from 0.75–0.86. These values changed very slightly to 0.79–0.86 over the test period. The pH of all samples increased slightly from 5.25–5.84 at the beginning of storage to 5.62–5.90 at the end of storage (Table 4). All sausage samples added with SL had slightly higher pH values as compared to the samples without SL. The microbial metabolism may cause the sausage pH to increase. Amines formed after decarboxylating of amino acids result in an increase of the pH.[23]

Table 3: Change of total microbial counts in the sausages added with some preservatives alone or in combination with sodium lactate during storage at 4°C

Treatments	Total microbial counts[a] (log cfu/g) ±SD				
	0 day	3 days	7 days	14 days	21 days
Control	7.60±0.57A[b]	7.80±0.54A	7.42±0.17A	7.93±0.67A	8.35±0.01A
BHT (0.02%)	6.93±1.5ABC	7.46±0.39A	7.39±0.44A	7.33±0.04AB	7.65±0.60A
SL (2.5%)	5.61±0.16BCD	5.61±0.16B	4.50±0.64CD	4.89±0.58C	4.25±0.92C
PE (0.1%)	7.33±0.95AB	7.42±0.59A	7.53±0.35A	7.63±0.21A	7.49±0.22A
PE (0.1%)+SL (2.5%)	5.55±0.49CD	5.25±0.66B	5.53±1.09CD	6.06±1.12C	4.20±0.13C
MVE_1 (0.1%)	7.26±0.76ABC	7.44±0.2A	7.39±0.55A	6.80±1.39ABC	7.24±0.34A
MVE_1 (0.1%)+SL (2.5%)	4.77±0.54D	4.84±0.65B	5.18±0.20CD	4.88±0.39C	5.48±0.33B
MVE_2 (0.1%)	7.66±0.48A	7.58±0.36A	7.37±0.27A	7.07±1.00AB	7.37±0.21A
MVE_2 (0.1%)+SL (2.5%)	7.00±1.02ABC	5.16±0.65B	5.82±0.94BC	5.44±0.86BC	4.58±0.31BC
RM (0.1%)	7.25±0.85ABC	6.97±1.05A	7.08±1.24AB	7.85±1.56A	8.30±0.50A
RM (0.1%)+ SL (2.5%)	5.09±0.30D	4.74±0.23B	4.26±0.08CD	4.91±0.29C	4.24±0.72C

[a]Data are mean of two replications.

[b]Means within a column with different letters are significantly different (P< 0.05).

Table 4: Change of pH in the sausages added with some preservatives alone or in combination with sodium lactate during storage at 4°C

Treatments	pH [a] ±SD				
	0 day	3 days	7 days	14 days	21 days
Control	5.50±0.03B[b]	5.54±0.12DE	5.61±0.02B	5.61±0.01C	5.66±0.04DC
BHT (0.02%)	5.56±0.04B	5.62±0.07BDC	5.67±0.07B	5.72±0.05B	5.76±0.04ABCD
SL (2.5%)	5.83±0.06A	5.84±0.04A	5.83±0.04A	5.84±0.08A	5.85±0.06ABCD
PE (0.%)	5.25±0.11C	5.37±0.01E	5.50±0.01C	5.53±0.01D	5.62±0.03D
PE (0.1%)+SL (2.5%)	5.76±0.04A	5.83±0.01A	5.81±0.05A	5.85±0.00A	5.85±0.03ABC
MVE_1 (0.1%)	5.45±0.11B	5.53±0.12DE	5.58±0.06BC	5.60±0.01C	5.70±0.08BCD
MVE_1 (0.1%)+SL (2.5%)	5.79±0.06A	5.79±0.08ABC	5.82±0.04A	5.84±0.02A	5.89±0.10BA
MVE_2 (0.1%)	5.48±0.11B	5.53±0.07DE	5.60±0.05BC	5.66±0.06BC	5.73±0.09ABCD
MVE_2 (0.1%)+SL (2.5%)	5.81±0.03A	5.81±0.06BA	5.82±0.04A	5.84±0.02A	5.89±0.13BA
RM (0.1%)	5.50±0.03B	5.60±0.04AC	5.61±0.06B	5.71±0.06B	5.76±0.02ABCD
RM (0.1%)+ SL (2.5%)	5.84±0.05A	5.84±0.05A	5.85±0.07A	5.85±0.07A	5.90±0.12A

[a]Data are mean of two replications.

[b]Means within a column with different letters are significantly different (P< 0.05).

Table 5: Change of color values in the sausages added with some preservatives alone or in combination with sodium lactate during storage at 4°C

Treatments	Color values[a] ± SD				
	0 day	3 days	7 days	14 days	21 days
L*-value					
Control	40.24±3.44A[b]	39.09±0.62A	41.23±6.42A	39.72±1.73A	37.84±0.69AB
BHT (0.02%)	41.81±4.07A	39.26±3.82A	40.76±1.82AB	41.93±1.18A	40.31±6.39A
SL (2.5%)	40.54±0.09A	37.70±0.72A	39.19±8.06AB	37.16±0.13ABCD	39.57±1.83A
PE (0.1%)	37.17±6.44A	36.18±3.92A	36.16±3.08B	34.90±4.64BCD	33.98±5.31AB
PE (0.1%)+SL(2.5%)	38.39±1.63A	35.86±3.68A	37.86±0.59AB	33.57±1.92D	35.59±6.26AB
MVE_1 (0.1%)	38.24±5.13A	35.14±0.73A	39.58±4.45AB	35.72±0.26BCD	33.40±1.27B
MVE_1 (0.1%)+SL (2.5%)	37.44±4.80A	38.66±3.60A	37.68±1.54B	36.42±1.99ABCD	38.33±2.26AB
MVE_2 (0.1%)	40.73±5.35A	40.87±5.02A	39.03±2.54AB	33.80±2.75CD	36.22±6.42AB
MVE_2 (0.1%)+SL (2.5%)	39.20±3.61A	34.72±4.19A	36.18±3.33B	33.64±4.22D	33.90±3.00AB
RM (0.1%)	41.48±1.87A	35.67±2.48A	37.83±0.03AB	38.02±0.86ABC	36.21±3.04AB
RM (0.1%)+ SL (2.5%)	40.66±6.63A	39.98±0.64A	40.55±2.22AB	39.58±2.59AB	37.84±5.71AB
A*-value					
Control	8.83±2.06A	8.95±0.93AB	5.59±1.80ABC	6.55±2.38AB	6.55±2.06A
BHT (0.02%)	8.16±1.62AB	6.64±0.08AB	5.99±1.20ABC	5.65±2.62AB	6.42±3.73A
SL (2.5%)	7.08±0.61ABC	8.30±1.77AB	7.76±0.91A	7.58±0.94A	6.20±0.16A
PE (0.1%)	6.51±0.24BD	5.52±0.60AB	5.76±0.81ABC	4.81±0.28AB	5.51±1.12A
PE (0.1%)+SL (2.5%)	4.48±0.89D	5.32±0.37A	3.79±1.06C	5.65±1.93AB	4.19±1.76A
MVE_1 (0.1%)	5.30±0.99CD	5.36±0.15AB	4.19±2.31BC	5.03±0.77AB	5.10±0.21A
MVE_1 (0.1%)+SL (2.5%)	5.14±0.28CD	4.26±0.40AB	4.18±0.25BC	4.21±0.11B	3.73±0.52A
MVE_2 (0.1%)	5.43±0.14CD	3.31±1.20B	4.71±0.31BC	5.47±0.05AB	4.56±0.39A
MVE_2 (0.1%)+SL (2.5%)	4.93±0.29CD	5.51±0.24AB	4.70±0.16BC	5.75±0.93AB	4.78±0.64A
RM (0.1%)	6.02±1.22BCD	8.55±0.66AB	6.87±1.68AB	6.12±0.42AB	5.76±0.81A
RM (0.1%)+ SL (2.5%)	6.10±1.38BCD	6.20±1.15AB	5.04±0.01ABC	4.83±1.27AB	5.28±0.86A
B*-value					
Control	4.55±0.96ABC	5.40±0.33A	5.69±0.88ABCD	5.40±0.86A	4.32±1.63A
BHT (0.02%)	4.47±1.07ABC	3.88±0.10A	5.80±0.75ABCD	5.88±1.26A	4.34±0.57A
SL (2.5%)	3.43±1.22ABC	4.11±0.19A	4.47±0.40D	3.31±0.71A	4.19±0.44A
PE (0.1%)	4.65±2.01AB	4.58±0.74A	4.28±0.36BCD	3.27±0.01A	4.77±0.76A
PE (0.1%)+SL (2.5%)	2.69±2.12BC	3.02±0.42A	5.20±1.16BCD	2.99±2.82A	5.09±0.38A
MVE_1 (0.1%)	3.14±0.86ABC	4.46±1.88A	8.00±1.18A	5.91±1.75A	3.64±1.36A
MVE_1 (0.1%)+SL (2.5%)	2.65±0.28ABC	5.88±0.88A	5.14±2.52BCD	6.47±2.97A	3.55±0.26A
MVE_2 (0.1%)	4.79±0.28A	6.10±2.91A	7.52±0.58ABC	4.93±0.86A	3.30±2.63A
MVE_2 (0.1%)+SL (2.5%)	3.23±0.81ABC	6.09±0.47A	7.24±0.36AB	3.86±0.71A	4.48±1.06A
RM (0.1%)	4.45±0.06ABC	5.17±1.07A	6.29±1.32BCD	6.56±0.19A	2.74±1.29A
RM (0.1%)+ SL (2.5%)	3.41±1.18ABC	6.35±1.14A	4.55±1.35CD	4.31±0.16A	4.39±0.40A

[a]Data are mean of two replications.

[b]Means within a column with different letters are significantly different (P< 0.05).

For color values, L* values (lightness) of almost all samples decreased throughout the 21-day storage (Table 5). Among all treatments, the lowest change of L* values were found in the samples added with BHT and those added with sodium lactate after 21 days of storage. For a* value (redness), the results showed that all treatment samples added with natural additives had lower initial a* values (4.48–6.51) than the samples with no addition of natural additives (7.08–8.83). Generally, the a* values of all sausages gradually decreased during storage. At the end of storage, a* values of the sausages were ranging from 3.73–6.55, but no significant difference was found between a* value of each treatment (P>0.05). However, b* value (yellowness) of most samples tended to increase as the storage time increased, except for the control sample and the samples added with BHT, MVE_2 and RM which showed a decrease of this color value after 14 days of storage. At the end of storage, L*, a* and b* values of the samples changed (from 37.17–41.48, 4.48–8.83 and 2.65–4.65) to 33.40–40.31, 3.73–6.55 and 2.74–5.09, respectively. Decreasing L* and a*values may relate to oxidative deterioration of meat lipid. Gordon[3] stated that lipid oxidation may cause bleaching of foods due to reaction of meat pigments with the reactive intermediates, free radicals.

Sensory characteristics

The results of sensory evaluation revealed that the only sensory attribute which provided useful information for treatments of different preservatives was rancid odor. After 21 days of storage, the control sample had higher rancidity (score of 2.72, moderately rancid odor) compared to freshly prepared sausage while other treatment samples received lower scores of rancid odor ranging from 1.38–2.25 (less rancid odor). The sausage added with 0.1% PE in combination with 2.5% SL received the lowest score of rancid odor (Figure 2). The difference of other sensory attributes (color, texture, sweetness and acceptability) were not found between each treatment.

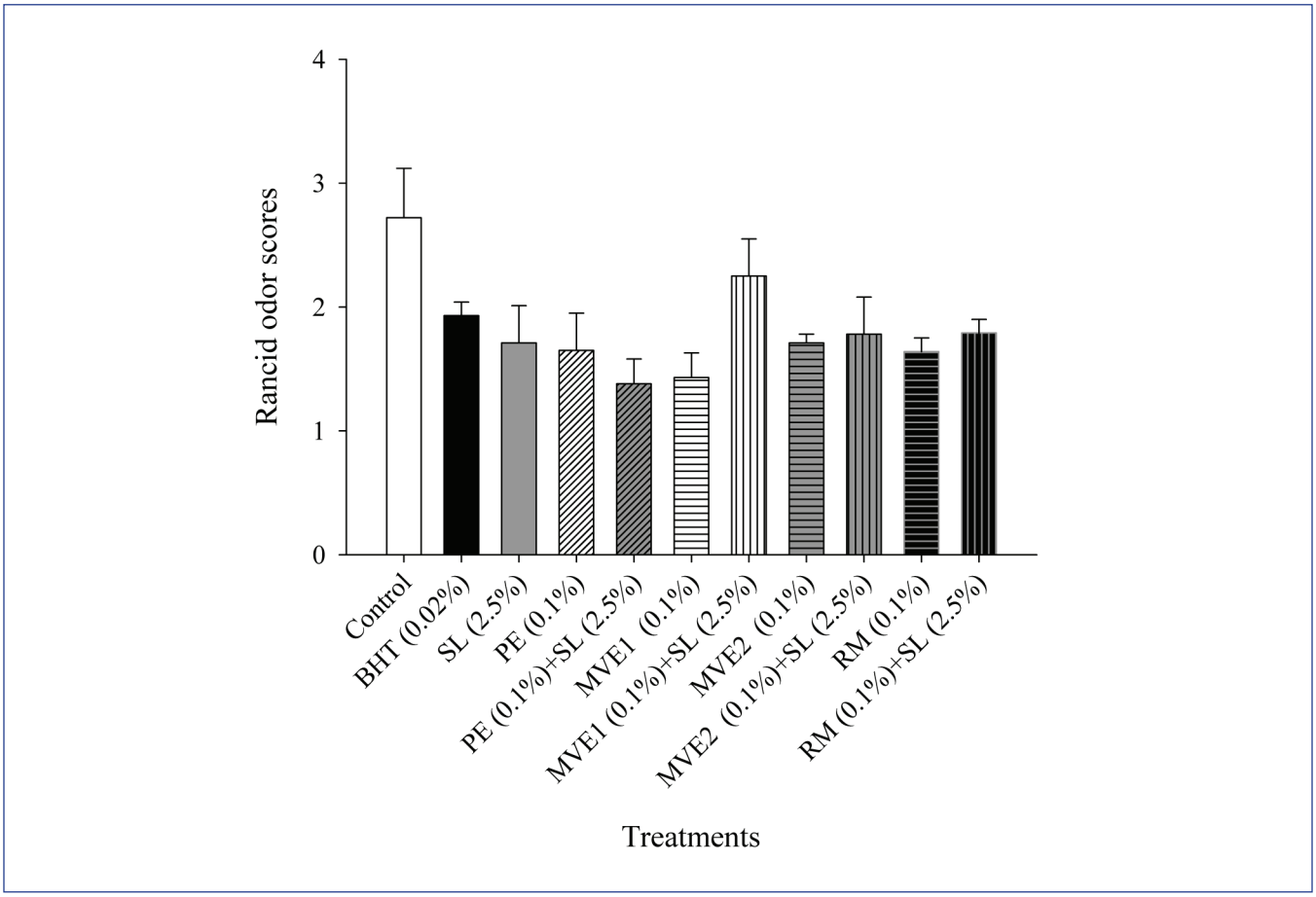

Figure 2: Rancid odor scores of the sausages added with some preservatives alone or in combination with sodium lactate after 21 days of storage at 4°C.

In conclusion, 0.1% concentration of Thai local vegetable extracts, especially *P. odoratum* extract could effectively be used as a natural preservative to delay lipid oxidation in high-fat meat products and should be used in combination with 2.5% sodium lactate for inhibition of microbial growth during storage.

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